these solutes is consistent with an osmotic stress effect, i.e., with their effect on the chemical potential of water modulating the equilibrium between folded and unfolded states via protein differential hydration. Moreover, the free energy of protein folding depends on solute size. We show that the apparent difference in hydration between molten globule and unfolded states of apo-Mb increases linearly with increasing solute sizes, while the free energy change of protein hydration upon folding decreases with the inverse of molar solute apparent volume. We have analyzed these size effects considering the contribution of excluded volume interactions to protein folding via Monte Carlo simulations of a self-avoiding walk chain in a cubic lattice with hardcore solutes. It is shown that solute-chain self-avoiding interaction decrease the conformational entropy of the chain in proportion to its square radius of gyration, and to the solute volume fraction occupied by the solute. The computational results translate into a difference in the chemical potential between folded and unfolded protein states that remarkably predicts the experimental influence of solute sizes and water chemical potential on the free energy change of apo-Mb refolding induced by osmolytes. Thus, this work may stablish a quantitative link between protein hydration and protein excluded-volume interactions and their effect on the energetic of protein folding.

280-Pos Board B66
A Partially Structured Molten Globule Protein
Joerg Reichenwallner, Mohammed Chakour, **Wolfgang E. Trommer**.
Kaiserslautern Technical University, Kaiserslautern, Germany.
Maltose binding protein (MBP) from *E. coli* was shown to bind maltose even in its molten globule state, although with substantially reduced affinity. The native protein of which the X-ray structure is known, is devoid of cysteines. Seven different mutants with two cysteines each were labeled with the MTS-SL. Distances from the active site as derived from the X-ray structure vary from 14 to 31 Å. DEER measurements have so far shown very good agreement between the X-ray data and the native structure. Here we compare distances in the native protein with those in the molten globule state.

281-Pos Board B67
Collagen Unfolding Determines Fluid Transfer in the Interstitial Matrix
Maria P. McGee, Michael Morykwas, Louis Argenta.
Wake-Forest University Medical School, Winston-salem, NC, USA.
Local unfolding of collagen at physiologic temperatures is thought to facilitate interactions with enzymes and scaffold molecules during inflammation, tissue remodeling, and wound healing. Previous data showing high interstitial hydration potential (HP) in human and porcine dermis after collagen thermal unfolding and fibroblast death suggest that it also plays a role in local modulation of interstitial flows. To test this hypothesis, collagen was progressively unfolded *in situ*, and changes in HP and water influx-rate within the matrix were measured as a function of the extent of unfolding, which was quantified by differential scanning calorimetry in full-thickness dermal samples after timed heat-treatment at 60°C and equilibration at 4°C. HP was determined by osmotic stress techniques, and influx-rates from time-dependent gravimetric changes under 35mmHg osmotic counterpressure. Both increased linearly with the proportion of unfolded collagen: the HP by 1.08 ± 0.16 mmHg, and the influx-rate by 3.19 ± 0.39 µl/min /100g per each 1% of collagen unfolded (R^2= 0.93 and 0. 95, respectively). The relative humidity and intensity of T2-weighed magnetic resonance images of the dermis also increased with the extent of collagen unfolding, confirming interfacial energy contributions to the HP - as predicted by the Kelvin relationship - and the expected hydrophobic nature of the newly formed protein/water interfaces, respectively. These results are fully consistent with the hypothesis and point to yet another potentially important function of local collagen unfolding in tissue homeostasis. As a plausible mechanism for HP and influx-rate increases with collagen unfolding, we propose that the surface tension of vapor/water interfaces under exposed hydrophobic clusters is higher than at hydrophilic interfaces; at nanometer scales, these differences generate local surface-tension gradients in the matrix that accelerate water influx and shift the HP.

282-Pos Board B68
Temperature Dependence of Protein Folding in Live Cell
Minghao Guo, Martin Gruebele.
University of Illinois - Urbana Champaign, Urbana, IL, USA.
Protein folding kinetics is known to be non-Arrhenius temperature dependent. We use Fast Relaxation Imaging (FReI) to measure stability and folding kinetics of FRET-labeled destabilized phosphoglycerate kinase (PGK). With modulated heating laser, we are able to measure the thermodynamics of PGK rapidly across the midpoint of transition of protein unfolding to minimize baseline shifts coming from photobleaching and protein aggregation.
We have measured PGK folding kinetics from 295K to 320K both in vitro and in vivo. Kinetics of PGK as mutiplestate folder can be fitted to stretched exponential. Folding rate and folding mechanism of PGK are correlated and both are strongly dependent on temperature, which can be explained by solvent viscosity and hydrophobic interactions.

283-Pos Board B69
Folding Mechanism of a Precursor Protein of a Peptide Hormone Mediated by an Intra-Molecular Chaperone
Masaki Okumura[1], Yu-ichiro Yoshida[2], Hiroshi Yamaguchi[1], Yuji Hidaka[2].
[1]Kwansei Gakuin univ., Sanda, Japan, [2]Kinki univ., Higashi-osaka, Japan.
Prouroguanylin is a precursor of uroguanylin. The mature form of uroguanylin contains intra-molecular disulfide bonds (Cys74-Cys82 and Cys77-Cys85). The propeptide region functions as an intra-molecular chaperone in the formation of the native conformation and the disulfide pairings of uroguanylin. To elucidate the mechanism of the propeptide-mediated folding, the pathway associated with the disulfide-coupled folding of prouroguanylin was examined in detail.
Prouroguanylin, when prepared using an *E. coli* expression system, was obtained as an inclusion body. Therefore, it was purified as a reduced/denatured protein by reversed-phase HPLC after solubilization in urea. The folding reaction was carried out 0.1 M Tris/HCl (pH 8.0) at various concentrations of glutathione in the presence and absence of protein disulfide isomerase which catalyzes the disulfide exchange reaction.
Kinetic analyses of the oxidative folding revealed that two types of intermediates containing mis-bridged disulfide bonds (namely, isomers 1 and 2 in which the disulfide bonds were between Cys74-Cys85 and Cys77-Cys82 and Cys74-Cys77 and Cys82-Cys85 in the mature region, respectively) are predominantly included in the folding. However, only one type of intermediate containing mis-disulfide bonds, isomer 2, was able to proceed to the native conformation of prouroguanylin, regardless of the presence of protein disulfide isomerase.
The results of these experiments will be discussed in this presentation.

284-Pos Board B70
Role of Leu66 in the Folding of Uroguanylin Assisted by Intra-Molecular Chaperone
Yu-ichiro Yoshida[1], Masaki Okumura[2], Shigeru Shimamoto[1], Hiroshi Yamaguchi[2], Yuji Hidaka[1].
[1]Kinki University, Higashi-Osaka, Japan, [2]Kwansei Gakuin University, Sanda, Japan.
Uroguanylin is maturated via the processing of a precursor protein, prouroguanylin. The pro-peptide region of the precursor protein of uroguanylin regulates the formation of the native structure of uroguanylin, by serving as an intra-molecular chaperone. To estimate the role of the individual amino acid residues of the pro-peptide region in chaperon function, we previously prepared Gly or Ala mutants and the folding of the mutant proteins were examined. The results revealed that, except for Cys residues, only the Leu66 residue critically affected the folding of the mature region, uroguanylin. To further investigate the role of the Leu66 residue in the folding of uroguanylin, it was mutated to several different amino acid residues, such as Gly, Ala, Val, and Ile.
The cDNA's encoding the mutant proteins were amplified by polymerase chain reaction and inserted into pET17b vector. The mutant proteins were expressed using the T_7-promoter expression system in *E. coli* BL21(*DE3*) cells. The mutant proteins were obtained as inclusion bodies and solubilized in 0.1 M Tris/HCl (pH 8.0) containing 8 M urea and dithiothreitol. The reduced forms of the mutant proteins were purified by reversed-phase high performance liquid chromatography (HPLC) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analyses.
The oxidative folding of the mutant proteins was carried out in the presence of reduced and oxidized forms of glutathione and the progress monitored by HPLC.
The results of these experiments will be discussed in this paper.

285-Pos Board B71
Evaluating a Key Player in Acute Heart Failure: Interaction Surfaces and Structural Details of Interleukin-33
Kaitlin Fisher.
UCSD, San Diego, CA, USA.
The newest member of the Interleukin-1 family of proteins is IL-33. IL-33 was recently discovered in 2005 and since then has been identified as a key participant in immune and inflammatory responses through association with the IL-1 receptor family member ST2. However, the structural homology between IL33 and other members of the interleukin family are low- presenting unique sequence identity, unique receptor interactions, and potentially unique signaling mechanisms associated with its activity. The current, limited understanding of